

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. **(Currently Amended)** A method for determining the presence of living bacteria or fungus-yeast in a sample by detecting ribonucleic acid (RNA) that comprises a selected target region of ribosomal RNA (rRNA) in a sample suspected of containing said bacteria and/or fungus, wherein said RNA comprises a selected target sequence, said method comprising:

(a) providing a extracting ribonucleic acid (RNA) from said sample to be tested or which is suspected of containing particular bacteria or fungus-yeast RNA;

(b) incubating said RNA from said sample with DNase;

(b c) incubating the bacteria or fungus-yeast said RNA from said sample with a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity and polynucleotide primers that hybridize to the selected rRNA target region of bacteria or fungus-yeast but not of other organisms, under conditions which allow the Reverse Transcriptase activity of said thermostable enzyme to synthesize cDNA from said target region the RNA target sequence; and said DNA polymerase activity to amplify said cDNA amplifying the cDNAs formed to a detectable level by Polymerase Chain Reaction with said DNA polymerase activity of the thermostable enzyme and polynucleotide primers and;

(e d) detecting the amplified cDNAs from said rRNA target region by hybridization with one or more probe polynucleotide(s) that hybridizes to said amplified cDNAs of bacteria or fungus-yeast but not of other organisms, wherein step (c) and (d) are performed in the same tube by means of one step real time RT-PCR.

2. **(Cancelled)**

3. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probe consist of the sequences:

Seq ID No 1 TGGAGCATGTGGTTTAATTCGA [primer forward]
Seq ID No 2 TCGGGACTTAACCCAACA [primer reverse]
Seq ID No 11 TGCATGGYTGTCGTCAGCTCGTG [probe forward].

4. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probe consist of the sequences:

Seq ID No 3 AGAGTTTGATCATGGCTCAGA [primer forward]
Seq ID No 4 TTACCCACCTACTAGCTAAT [primer reverse]
Seq ID No 12 GAGTGGCGGACGGGTGAGTAA [probe forward]

5. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probe consist of the sequences:

Seq ID No 5 GYGGAGCATGTGGYTTAATTCG [primer forward]
Seq ID No 6 TTGCGCTCGTTRCGGGACTT [primer reverse]
Seq ID No 13 ACAGGTGGTGCATGGTTGTC [probe forward]
Seq ID No 14 TCAGCTCGTGTGTCGAGATGTT [probe forward]
Seq ID No 15 ACAGGTGCTGCATGGCTGTC [probe forward]
Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT [probe forward].

6. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probe consist of the sequences:

Seq ID No 7 GGGAAACTCACCAGGTCCA [primer forward]
Seq ID No 8 CGTTATCGCAATTAAGCAGACA [primer reverse]

Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT [probe forward] .

7. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probe consist of the sequences:

Seq ID No 9 GGTAACGGGGAATWAGGGTTC [primer forward]

Seq ID No 10 TTGGGTAATTTGCGCGCCTG [primer reverse]

Seq ID No 18 CGGAGAGGGAGCCTGAGAA [probe forward]

Seq ID No 19 CGGCTACCACATCCAAGGAA [probe forward] .

8. **(Previously presented)** The method of claim 1 wherein the primers and probes consist of the sequences:

Seq ID No 1+ Seq ID No 2 +Seq ID No 11 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 3+ Seq ID No 4 +Seq ID No 12 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 5+ Seq ID No 6 +Seq ID No 13 + Seq ID No 14 + Seq ID No 15 +Seq ID No 16 + Seq ID No 9+Seq ID No 10 +Seq ID No 18 +Seq ID No 19.

9. **(Previously presented)** The method of claim 1 wherein the polynucleotide primers and probes are natural nucleic acid or Peptide Nucleic Acid (PNA) which can hybridize to nucleic acid (DNA and RNA).

10. (Previously presented) The method of claim 1 further comprising the step of quantifying the RNA by comparison with a quantified external standard RNA from the group consisting of : *Escherichia coli* and *Candida spp.*

11. (Currently amended) The method of ~~claims 1 or 2~~ claim 1 wherein step (a) comprises extracting bacteria or fungus-yeast RNA from the sample ~~up to 1000ml~~ by ~~centrifiltration~~ centrifiltration on membranes and/or DEAE resin ~~followed by incubation with DNase.~~

12. (Cancelled)

13. (Currently amended) The method of ~~any one of claims 1 to 4~~ claim 1 wherein the thermostable enzyme is *Tth* DNA polymerase.

14. (Cancelled)

15. (Currently amended) The method of claim ~~14~~ 1 wherein the polynucleotide primer(s) for synthesizing cDNA by Reverse Transcription are selected from the group consisting of:

Seq ID No 2	TGCGGGACTTAACCCAACA	[primer reverse]
Seq ID No 4	TTACCCACCTACTAGCTAAT	[primer reverse]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer reverse]

Seq ID No 10 TTGGGTAATTTGCGCGCCTG [primer reverse] .

16. (Currently amended) The method of claim 14 1 wherein the polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction are selected from the group consisting of:

Seq ID No 1	TGGAGCATGTGGTTTAATTCGA	[primer forward]
Seq ID No 2	TGCGGGACTTAACCCAACA	[primer forward]
Seq ID No 3	AGAGTTTGATCATGGCTCAGA	[primer forward]
Seq ID No 4	TTACCCACCTACTAGCTAAT	[primer forward]
Seq ID No 5	GYGGAGCATGTGGYTAAATTCG	[primer forward]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer forward]
Seq ID No 7	GGGAAACTCACCAGGTCCA	[primer forward]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer forward]
Seq ID No 9	GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer forward] .

17. (Currently amended) The method of claim 14 1 wherein the polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs is/are selected from the group consisting of:

Seq ID No 11	TGCATGGYTGTCGTCAGCTCGTG	[probe forward]
Seq ID No 12	GAGTGGCGGACGGGTGAGTAA	[probe forward]
Seq ID No 13	ACAGGTGGTGCATGGTTGTC	[probe forward]
Seq ID No 14	TCAGCTCGTGTCGTGAGATGTT	[probe forward]
Seq ID No 15	ACAGGTGCTGCATGGCTGTC	[probe forward]
Seq ID No 16	TCAGCTCGTGTTGTGAAATGTT	[probe forward]
Seq ID No 17	AGGATTGACAGATTGAGAGCTCTT	[probe forward]

Seq ID No 18 CGGAGAGGGAGCCTGAGAA [probe forward]

Seq ID No 19 CGGCTACCACATCCAAGGAA [probe forward] .

18. **(Currently amended)** The method of claim 9 wherein the polynucleotide probes further ~~comprise~~ comprise a non-radioactive label.

19. **(Previously presented)** The method of claim 18 wherein the non-radioactive label is a fluoroscein.

20. **(Withdrawn)** A kit for determining the presence of bacteria or fungus-yeast ribonucleic acid (RNA) in a sample suspected of containing said bacteria and/or fungus comprising:

- (a) a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity;
- (b) polynucleotide primers comprising:
 - (i) a polynucleotide primer or polynucleotide primers for synthesizing cDNA by Reverse Transcription;
 - (ii) polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction; and
 - (iii) a polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs.

21. **(Withdrawn)** The kit of claim 20 further comprising centrifiltration membranes and/or DEAE resin for obtaining bacteria or fungus-yeast RNA from a sample.

22. **(Withdrawn)** The kit of claim 20 further comprising DNase.

23. **(Withdrawn)** The kit of any one of claims 20 to 22 wherein the polynucleotide primers for synthesizing cDNA by Reverse Transcription are selected from group consisting of:

Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]

Seq ID No 4	TTACCCACCTACTAGCTAAT	[primer reverse]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer reverse]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer reverse].

24. **(Withdrawn)** The kit of any one of claims 20 to 22 wherein the polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction are selected from the group consisting of:

Seq ID No 1	TGGAGCATGTGGTTTAATTCGA	[primer forward]
Seq ID No 2	TGCGGGACTTAACCCAACA	[primer forward]
Seq ID No 3	AGAGTTTGATCATGGCTCAGA	[primer forward]
Seq ID No 4	TTACCCACCTACTAGCTAAT	[primer forward]
Seq ID No 5	GYGGAGCATGTGGYTAAATTCG	[primer forward]
Seq ID No 6	TTGCGCTCGTTRCGGGACTT	[primer forward]
Seq ID No 7	GGGAACTCACCAGGTCCA	[primer forward]
Seq ID No 8	CGTTATCGCAATTAAGCAGACA	[primer forward]
Seq ID No 9	GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10	TTGGGTAATTTGCGCGCCTG	[primer forward].

25. **(Withdrawn)** The kit of any one of claims 20 to 22 wherein the polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs is/are selected from the group consisting of:

Seq ID No 11	TGCATGGYTGTCGTCAGCTCGTG	[probe forward]
Seq ID No 12	GAGTGGCGGACGGGTGAGTAA	[probe forward]
Seq ID No 13	ACAGGTGGTGCATGGTTGTC	[probe forward]
Seq ID No 14	TCAGCTCGTGTCGTGAGATGTT	[probe forward]
Seq ID No 15	ACAGGTGCTGCATGGCTGTC	[probe forward]

Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT [probe forward]

Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT [probe forward]

Seq ID No 18 CGGAGAGGGAGCCTGAGAA [probe forward]

Seq ID No 19 CGGCTACCACATCCAAGGAA [probe forward] .

26. **(Withdrawn)** The kit of any one of claims 20 to 22 wherein the thermostable enzyme is
Tth DNA polymerase.

27. **(Withdrawn)** The kit of any one of claims 20 to 22 for performing a method as defined
in Claim 1.

28. **(Cancelled)**

29. **(Cancelled)**